Total Hg and methylmercury dynamics in a river-floodplain system in the Western Amazon: Influence of seasonality, organic matter and physical and chemical parameters

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**HIGHLIGHTS**

• Alluvial floodplains in the Amazon region have a complex Hg biogeochemical cycle.
• Floodplains had higher Hg and MeHg concentrations relative to Madeira River.
• δ13C-isotope and (C:N) ratios were useful for predicting MeHg concentrations.
• Total Hg showed higher affinity for the SPM than for the dissolved matter.
• Aquatic macrophyte and sediment MeHg demonstrated transfer from sediment to biota.

**GRAPHICAL ABSTRACT**

**ABSTRACT**

Total mercury (Hg) and methylmercury (MeHg) circulation in a connected river-floodplain system composed of two black water (a small forest river, igapô, and Cuniã Lake) and a white water body (Madeira River), located in the Madeira River Basin were evaluated during the rising-water, early and late falling-water periods. We assessed organic matter (C and N composition, (C:N)ε; and δ13C isotopic signature), and physical and chemical influences (pH, dissolved O2, electric conductivity) in relationship to Hg and MeHg concentrations. Hg and MeHg concentrations in a sediment profile and three aquatic macrophytes (E. crassipes, E. azuera and Oryza sp.) were measured. Igapô and Cuniã Lake showed higher Hg and MeHg concentrations (115–709; 10–25 ng g⁻¹) in the suspended particulate matter compared to the Madeira River (Hg: 5–16; MeHg: 0.2–0.3 ng g⁻¹), partially independent of seasonality (p = 0.06). Total Hg had higher affinity for the SPM (1.75 times) than for dissolved organic matter. Organic matter characteristics correlated with MeHg concentrations (δ13C and (C:N)ε; r² = 0.79; p < 0.0001), as well as physical and chemical parameters of the water column (dissolved O2 and pH; r² = 0.80; p < 0.0001), demonstrating that physical and chemical changes between the river-floodplain system affect MeHg circulation and production. The inverse correlation of MeHg and SO4²⁻ concentrations (r² = 0.73; p = 0.0001) suggests the action of sulfate-reducing bacteria. Total Hg and MeHg concentrations as well as MeHg were detected in the sediment profile (Hg: 24–51; MeHg: 0.6–3.2 ng g⁻¹; %MeHg: 1.8–6.2) and aquatic macrophytes (Hg: 1–30; MeHg: 0.3–7.5 ng g⁻¹; %MeHg: 1.6–33.7). We conclude that the highest Hg and MeHg concentrations in Cuniã Lake, compared to the Madeira River, are due to the physical and chemical differences between these environments.

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1. Introduction

Alluvial floodplains, which occur widely in the Amazon region, play an important role in the Hg biogeochemical cycle (Maia et al., 2009; Maia et al., 2017; Brito et al., 2017). They exchange water with the large rivers to which they are connected to some extent all year, specifically during the rising-water and the falling-water periods. Floodplains act as a major sink and source of water to the main rivers, which promotes physical and chemical modifications (Sioli, 1967; Bittencourt and Amadio, 2007). These modifications directly affect Hg methylation, and Hg and MeHg partitioning between the particulate and dissolved fractions of the water column (Maurice-Bourgoin et al., 2007; Maia et al., 2009).

Some floodplains form lakes characterized by high primary production, estimated at 106 tC hec⁻¹ year⁻¹, of which 73% is related to phytoplankton and aquatic macrophytes and the remaining 27% is related to the adjacent flooded forest (Junk and Furch, 1985). This creates a difference in the organic matter concentration between these lakes and the rivers to which they are connected. During the low-water period, the lower connectivity between these environments and their hydrodynamical differences partially limit the autochthonous processes of lakes to be diluted by allochthonous entries from rivers, allowing a maximum physical and chemical parameters amplitude between these two environments (Junk, 1989; Lacerda et al., 1989; Richey et al., 2002).

Sioli (1967) classified the Amazonian water bodies into large groups based on their color, e.g. clear, white and black-water bodies. White water rivers, like Madeira and Solimões, have neutral pH and high concentrations of ions and suspended particulate matter (SPM), eroded from the Andean region. Black water rivers, like the Negro and some igarapés (small streams, especially those surrounding by forest), drain areas that already have very eroded soils and are characterized by high concentrations of dissolved organic carbon, low content of ions and SPM and slightly acid pH (Junk and Furch, 1985). Moreira-Turcq et al. (2003) studied the mixture of waters at the Negro and Solimões confluence (also studied by Kasper et al., 2017), verifying large adsorption of the dissolved organic matter from the Negro River on the SPM present in the Solimões River. They verified that approximately 40% of the organic matter can be removed from the dissolved fraction through this process and that the SPM, now possessing organic and inorganic binding sites, easily adsorbs and transports Hg, as already reported in the literature (Bezerra et al., 2009). Hg mobility can be influenced by: (1) the association of organic matter with the SPM that can be deposited and immobilize Hg, or (2) the formation of stable Hg complexes with organic matter in the dissolved fraction, which increases the availability of the contaminant to the biota (Bäckström et al., 2003; Hammerschmidt et al., 2004).

Given the close relationship between organic matter and Hg, several authors have used the elemental and isotopic signatures of C and N as proxies to identify the contribution of different sources of the contaminant to aquatic ecosystems (Campbell et al., 2006; Stewart et al., 2008; Cremona et al., 2009; Jara-Marini et al., 2012). These sources can be divided into five: two allochthonous sources (debris from land plants and soils); two autochthonous sources (debris from the decomposition of aquatic macrophytes and algae), and a last source formed by bacteria and fungi, whose characteristics are linked to the mixture of the first two groups (Sulzman, 2007; Martinielli et al., 2003). The isotopic signature of δ¹³C (δ¹³C in the SPM of a black water river (Negro River) is lighter than the signature of a white-water river (Madeira River), due to the different types of soils they drain and the autotrophic – heterotrophic dynamics occurring in their respective water columns. In the same sense, the atomic and elemental ratio between carbon and nitrogen (abbreviated by (C:N)n) exhibited higher values in the Negro River compared to the Madeira and Tapajós rivers, suggesting that the organic matter of black water rivers is composed of debris of vascular terrestrial plants (Hedges et al., 1986, 1994; Pinheiro et al., 2014). On the other hand, the low (C:N)n ratio found in white water rivers and clear water rivers (rivers with low amounts of SPM, conductivity between 10 and 20 μS cm⁻¹, greenish waters and pH between 6 and 7; Sioli, 1967) may be a reflection of the microbial biomass, like phytoplankton and bacteria (Devol and Hedges, 2001).

Organic matter produced in floodplains by phytoplankton and aquatic macrophytes are mostly labile (i.e. low (C:N)n ratio; Junk and Furch, 1985; Quay et al., 1992) and, therefore, play an important role in the formation of methylmercury (CH₃Hg⁺, abbreviated by MeHg), a toxic chemical species of Hg. Quay et al. (1992) estimated that 40% of dissolved organic carbon present in the Amazon River is produced in floodplain lakes. The decomposition of organic matter generates an acidic, anoxic and reducing environment, and under these conditions some bacteria, such as sulfate-reducing bacteria, add a methyl group to Hg (King et al., 1999; Benoit et al., 2002). MeHg can be absorbed directly from water (by aquatic macrophytes and plankton) and in the diabetic tract of other animals that feed on organisms containing MeHg (Wiener et al., 2002). Guimarães et al. (2000) reported a 30-fold higher Hg methylation potential in the periphyton associated with aquatic macrophyte roots than in the underlying sediments. In floodplains, these plants represent approximately 60% of the total carbon production of the ecosystem, functioning as an important link for MeHg transfer to the trophic chain (Mauro et al., 1999).

Hg dynamics associated with the mixture of black and white waters are best studied at a small scale, since many Amazonian systems have a connection between rivers and floodplains (Junk, 1989; Gadel et al., 2000; Bonnet et al., 2008). These smaller systems have advantages as study models, since (1) they are present throughout the Amazon region; (2) they are more accessible for field studies and, (3) because they have different connectivity levels with the main rivers during the hydrological periods of the region, they make it possible to evaluate the influence of seasonality on Hg dynamics (Sioli, 1967; Lacerda et al., 1989; SEDAM, 2012).

In this sense, the aim of this study was to evaluate total Hg and MeHg circulation in the water column of black water (igarapé and Cuniã Lake) and white water bodies (Madeira River) along three hydrological periods characteristic of the region, accessing the influence of organic matter, and physical and chemical parameters differences in the Hg and MeHg associations. Our hypotheses were (1) the highest total Hg concentrations in the SPM from floodplain ecosystems (igarapé and Cuniã Lake) will be observed during the rising-water period, since erosion of particles from both Madeira River and the soil adjacent to the Cuniã igarapé is favored when the water levels of the river are high and (2) MeHg concentrations will be higher during the late falling-water period, when the influence of the Madeira River in the floodplain is smaller, due to its lower water level, and intense organic matter decomposition, generating physical and chemical conditions that favors Hg methylation.

2. Materials and methods

2.1. Study area

Cuniã Lake (Fig. 1) has a large and still conserved area, estimated at 180 km², representing about 1/3 of the total area of the extractive reserve of Cuniã Lake, and its water mostly comes from the Cuniã igarapé (Fig. 1), which connects the lake to the Madeira River (Fig. 1). Other smaller igarapés next to sample point Lake3 (Fig. 1) and runoff also contribute as water sources to Cuniã Lake during the region’s rainy season (INMET, 2018; Supplementary material 1). The maximum and minimum depth recorded during the sampling campaigns was 11 and 6 m during the rising-water and late falling-water period, respectively (Supplementary material 2). The reserve is located 150 km from the city of Porto Velho in the state of Rondônia (Brazil). The geomorphology consists of alluvial plains surrounded by fluvial terraces, whose altitudes do not exceed 100 m. The area has soil types that typically occur in regions with excess water (seasonal or permanent), but with precarious
drainage and a marked $O_2$ deficit, which usually restricts plant growth. These soils contain excess iron hydroxide, as a consequence of the state of almost permanent reduction, which impairs the growth of species that are flood-intolerant (ICMBIO, 2008).

Igarapés are small streams typical of the Amazon region. They can connect large rivers, such as the Madeira River, with its respective floodplain and its associated lakes. Cuniã $Igarapé$ is a small black water stream enclosed by forest that has a winding path of 37 km (Fig. 1) with maximum and minimum depth of 13 and 12 m recorded during the rising-water and late falling-water period, respectively (Supplementary material 2) and maximum width of 120 m. During the low-water period water stagnates in the most central portion, although the connection with the Madeira River is maintained. Autochthonous production directly increases the organic matter concentration in the watercourse, promotes changes soil and sediment characteristics through the action of litter decomposition, facilitating the cation exchange processes in disaggregated particles. The environment has $pH$ variation from 4.7 to 6.0 that promotes $Hg$ mobility between SPM and the dissolved phase of the water column. In addition, the seasonal flood pulse of the Madeira River results in input to $Igarapés$ a large mass of detrital SPM (transport capacity: $-4.108 \, t \, SPM \, year^{-1}$) and disaggregated particles from adjacent flooded soils of the $Igarapés$, rich in biological material, carrying them to the lake (Sioli, 1967; Junk and Furch, 1985; Santos and Ribeiro, 1988; ICMBIO, 2008).

The Madeira River (Fig. 1) was severely impacted by $Hg$ due to gold mining activity in the 1980s, and in addition, soils have naturally high $Hg$ concentrations (Lacerda et al., 1989; Roulet and Lucotte, 1995). $Hg$ impacts on fish and riverine population continues to be detected until the present day (Bastos et al., 2006; Arrifano et al., 2018a, 2018b) suggesting that $Hg$ circulation in the region is currently due mainly to natural processes that continue to create risks for the local population, specially the riverine population, as reported by Arrifano et al. (2018a, 2018b). The Madeira River is considered the second largest river of the Amazon, with 1460 km extension. This river has a drainage area of 2.018 million km², estimated at 600 billion m³ year⁻¹ (SEDAM, 2012). The latosols can be observed in approximately 58% of the watershed (SEDAM, 2012).

2.2. Sampling

Sampling was carried out in the early falling-water, rising-water and late falling-water hydrological periods (May 2012, February 2013 and June 2013, respectively; Supplementary material 1). Two sample points in the Madeira River (Madeira1, Madeira2, Fig. 1), two sample points in the Cuniã $Igarapés$ (Igarapê1, Igarapê2, Fig. 1) and three sample points in Cuniã Lake were considered (Lake1, Lake2, Lake3, Fig. 1). At each point, 6 L of water was sampled, integrating the water column vertically with a Van Dorn bottle, and stored in pre-rinsed polyethylene bottles with water from the same sampled point. Before integrating the water column, its maximum depth was measured (Supplementary material 2). Then, 6 L of water were sampled: 2 L just above the maximum depth, avoiding sediment resuspension, 2 L at the average depth, and 2 L in the surface layer of the water column.

A sediment profile was sampled manually from the central part of Cuniã Lake (Lake2; Fig. 1) using a PVC tube during the late falling-water period (June 2013; Supplementary material 1). Three species of aquatic macrophytes were also sampled during this period: $Eichornia crassipes$, $Eichornia azurea$ and $Oryza$ sp. Only one sample of each macrophyte was analyzed.

The physical and chemical parameters of the water column (dissolved $O_2$, $pH$, electric conductivity and temperature) were measured with digital potentiometers (Oxi 3110, pH 3110 and Cond 3110, DIGIMED, São Paulo, Brazil, respectively) during each sampling campaign and the geolocation was registered with a GPS receiver (Trimble GeoExplorer, Model XTS500, California, USA). The water column depth was measured manually with a millimeter rope (Supplementary material 2), with the exception of Madeira River points, whose values were obtained from Schwatke et al. (2015) for the same sampling month and year as this work (Supplementary material 1).

2.3. Processing of water, plant and sediment samples

Suspended particulate matter (SPM) was obtained by filtering the water samples using previously dried (lyophilization) and weighted Whatman® GF/F filters (0.7 µm porosity) until saturation. The filtrate was acidified with HCl and kept refrigerated.
A 2 mL aliquot of the filtrate (<0.7 μm) was percolated in an organic matter solid phase extraction column (Bond Elut Plexa, Agilent, California, USA), previously activated with 6 mL of 99% methanol (Merck, Darmstadt, Germany). After percolation, elution was carried out also with 99% methanol (8 mL) to obtain a final extract (dissolved matter) for total Hg determination, besides the isotopic and elemental signature of the organic matter. It was not possible to carry out this analysis in the early falling-water period because this method was not optimized at the time.

The sampled aquatic macrophytes were separated into roots, stems, leaves, flowers and fruits (when present). All tissues were washed with ultrapure water (Millipore Milli-Q, France) in abundance to remove the coarse material. The sediment profile (N = 1) was sliced 2 by 2 cm and sieved in the <2 mm fraction.

Filters containing SPM, aquatic macrophyte tissues and sediment samples were lyophilized (FreezeDry System, Labconco, Model 7522900, Kansas City, USA) and stored dry until chemical measurements were carried out. After lyophilization, the filters were weighted again to measure the total mass of SPM retained. The dissolved matter was dried at room temperature using a constant stream of N2 to avoid sample contamination with aerosols.

2.4. Total Hg determination

Solubilization of lyophilized samples (0.5 g of SPM and sediment; 1 g of aquatic macrophyte tissues) followed the methodological protocol adapted from Santos et al. (2005), in the following steps: addition of HCl:KNO3 (3, 37.1:1, 65%) and KMnO4 (5%) in a microwave oven (Mars Express, CEM, Model 907.301, USA) during 25 min (10 min until it reached 95 °C, and 15 min with constant temperature of 95 °C and power of 1600 W). The final extract was filtered using Whatman® 40 paper and completed to 30 mL with ultrapure water (Millipore Mili-Q, France) in a volumetric flask. The solubilization of dissolved matter was performed in amber vials at 70 °C in a water bath using the same reagent proportions.

Total Hg determination was carried out in a mercury analyzer (QuickTrace, M-75000, CETAC, Nebraska, USA) as described by Bastos et al. (1998). Calibration was achieved using a six-point curve from 0.1 ng mL⁻¹ to 5 ng mL⁻¹ (Hg standard, Titrisol, Merck, Darmstadt, Germany). The determination coefficient (r²) acceptable for carrying out the analyses was >0.996. The detection limit for SPM and sediment was 0.4 ng g⁻¹ dry weight (wt.). The detection limit for aquatic macrophyte tissues was 1 ng g⁻¹ dry wt. The recovery of sediment and SPM samples was 93% (NIST 2702) and for aquatic macrophyte samples it was 90% (NIST 1515) and the coefficient of variation between analytical triplicates was <10%.

2.5. MeHg determination

MeHg determination in biotic (aquatic macrophyte tissues) and abiotic samples (SPM and sediment) followed the protocol described by Liang et al. (1994) and EPA 1630 (2001), respectively. Aliquots of 0.5 g (aquatic macrophyte tissues, sediment and SPM) were weighed in Teflon® tubes in which 5 mL of a 25% KOH/methanol solution was added to extract and solubilize MeHg. These samples were kept in an oven for 6 h at 70 °C, and were shaken every hour. At the end of 6 h, the samples were allowed to rest for 48 h in the dark to avoid MeHg photodegradation.

At the end of 48 h, MeHg determination was carried out. Amber vials received 30 μL of the solubilized sample, 200 μL of a 2 mol L⁻¹ acetic acid/sodium acetate solution (CH₃COOH/CH₃COONa) and 50 μL of 1% sodium tetraethylborate (NaBEt₄) and were filled with ultrapure water (Millipore Milli-Q model Integral A10, Molsheim, France) to 40 mL.

The determination was carried out using a gas chromatograph coupled to a cold vapor atomic fluorescence spectrometer (GC-CVAFS, MERX-M Automated Methyl Mercury Analytical System, Brooks Rand Labs, Seattle, WA, USA). Calibration was achieved using a seven-point curve from 0.5 ng mL⁻¹ to 1000 ng mL⁻¹ (MeHg standard, CH₃HgCl, Brooks Rand Labs, Washington, USA). The determination coefficient (r²) acceptable for carrying out the analyses was >0.999. The detection limit was 0.0017 ng g⁻¹ dry wt. and the recovery was 91 ± 9% for biotic samples (IAEA-140) and 96 ± 10% for abiotic samples (IAEA-356). The coefficient of variation between analytical triplicates was <15%.

2.6. Total Fe determination

Total Fe determination in SPM samples followed the protocol described by Pozebon et al. (2005).

The determination was carried out using an atomic emission spectrometer (ICP-AES, Liberty Series II, Varian, Australia). Calibration was achieved using a six-point curve from 0.2 μg mL⁻¹ to 20 μg mL⁻¹ (Fe standard, Titrisol, Merck, Darmstadt, Germany). The determination coefficient (r²) acceptable for carrying out the analyses was >0.999. The detection limit was 0.2 μg g⁻¹ dry wt and the recovery was 93% (NIST 2702). The coefficient of variation between analytical triplicates was <10%.

2.7. Anion determinations (PO₄³⁻, SO₄²⁻, NO₃⁻, NO₂⁻ and Cl⁻)

Aliquots (10 mL) of unfiltered water samples were placed in Teflon® tubes and autoclaved for 30 min at 1 atm for orthophosphate (PO₄³⁻) measurement. After autoclaving, 2 mL of a combined solution containing ascorbic acid (C₆H₈O₆), ammonium molybdate ([NH₄]₆MoO₄·4H₂O), sulfuric acid (H₂SO₄) and potassium antimony tartrate (C₆H₈K₂O₃Sb₂) in the concentrations and proportions described by Murphy and Riley (1962) was added to each tube and the determinations were carried out using a UV-visible spectrophotometer (UV-160A, UV-Visible Recording Spectrophotometer, Shimadzu, Japan) at 882 nm (Murphy and Riley, 1962). Calibration was achieved using a six-point curve from 0.5 ng mL⁻¹ to 100 ng mL⁻¹ (orthophosphate standard, Ocean Scientific International, Hampshire, United Kingdom). The determination coefficient (r²) acceptable for carrying out the analyses was >0.99. The detection limit was estimated as three times the standard deviation of the blanks multiplied by the calibration factor, resulting in values of 0.9 ng mL⁻¹ for orthophosphate. The coefficient of variation between analytical triplicates was <15%.

The nitrate (NO₃⁻), nitrite (NO₂⁻), sulfate (SO₄²⁻) and chloride (Cl⁻) levels were quantified using an ion chromatograph (Metrohm, Model 861, Advanced Compact IC, Switzerland), as described by Carmouze (1994). The elution base for nitrate and nitrite determination contained oxalic acid (H₂C₂O₄) and sodium chloride (NaCl), while for chloride and sulfate it contained calcium carbonate (Na₂CO₃) and sodium bicarbonate (NaHCO₃).

2.8. C and N elemental composition and ¹³C isotopic signature of organic matter

The carbon and nitrogen elemental composition of organic matter was determined using 1 mg of SPM and aquatic macrophyte tissues samples placed in tin (Sn) capsules. For the sediment, a 10 mg aliquot was used and for the dissolved matter a 100 μL aliquot was used. The determinations were carried out in an elemental analyzer (Flash 2000, Organic Elemental Analyzer, Thermo Scientific, Milan, Italy) and the atomic ratios of carbon and nitrogen (abbreviated by (C:N)α) were calculated.

The carbon-13 isotopic ratio (¹³C/C¹²C, abbreviated by δ¹³C) was determined using an interface coupled to the elemental analyzer (Conflow IV) which carries the samples to a mass spectrometer (Isotope Ratio Mass Spectrometer, IRMS, Thermo Scientific Milan, Italy). The results were expressed in relation to the Pee Dee carbonate formation (Belemnite) in parts per billion (‰), with precision of 0.1‰. The reproducibility for the same sample was 95% for all matrices (Meyers, 1994; Cloern et al., 2002; Kennedy et al., 2005).
2.9. Statistical analyses

The data were analyzed and the graphs were produced using the R software (R Core Team, 2018). The descriptive statistics used were the median and median absolute deviation (abbreviated by MAD), due to the asymmetry of most of the data. Two-way analysis of variance (two-way ANOVA) and analysis of covariance (ANCOVA) were calculated (aov and lm functions; R Core Team, 2018) and tests for multiple comparison of means were performed a posteriori in both cases for the categorical variables, assuming 95% certainty (TukeyHSD function; R Core Team, 2018). Where necessary, a maximum likelihood function (boxcox function, MASS package, Venables and Ripley, 2002) was used to transform the data to meet the ANOVA and ANCOVA requirements (linearity, normality, homoscedasticity and low leverage of residuals).

The ellipses in the 2D scatter plots represent the dispersion of data to one standard deviation from the centroid of the respective variables and were calculated assuming Normal distribution of the data (dataEllipse function, car package, Fox and Weisberg, 2011). The overlap between the ellipse areas was calculated (maxLikOverlap function, SIBER package, Jackson et al., 2011) and is presented in percentage.

Linear, exponential, logarithmic and multiple regressions were calculated (lm function; R Core Team, 2018) and the determination coefficient ($r^2$), $p$ values and the 95% confidence and prediction intervals (predict function, R Core Team, 2018) of the models were reported. Where necessary, a maximum likelihood function (boxcox function, MASS package, Venables and Ripley, 2002) was used to transform the data to meet the regression requirements (linearity, normality, homoscedasticity and low leverage of residuals). Multiple regressions were presented through their predicted regression planes (predict function, R Core Team, 2018; dcast function, reshape2 package, Wickham, 2007), adjusted in interactive 3D graphics (plot3d, surface3d, movie3d functions, rgl package; Adler et al., 2018).

3. Results

3.1. Physical and chemical parameters of the water column

Significant effects of the relative distance from the floodplain points to the Madeira River and of the hydrological periods (ANCOVA without interaction) were detected in the values of the physical and chemical parameters, with the exception of temperature, which presented constant values among the sampled points and hydrological periods (Fig. 2 and Supplementary material 2).

Spatially, dissolved O$_2$ ($r^2 = 0.25; p = 0.005$), electrical conductivity ($r^2 = 0.85; p < 0.000001$) and pH ($r^2 = 0.53; p = 0.000001$) presented exponential decrease of their values with increasing distance from the Madeira River towards the floodplain lake (Fig. 2).

Seasonally, pH and dissolved O$_2$ values were significantly higher during the late falling-water period when compared to the rising-water period ($p = 0.018$ and $p = 0.0002$, respectively; Supplementary material 2) and early falling-water period ($p = 0.0001$ and $p = 0.00002$, respectively; Supplementary material 2). Electrical conductivity values were marginally higher during the rising-water period when compared to the late falling-water period ($p = 0.04$; Supplementary material 2).

3.2. Suspended particulate matter

3.2.1. Total Hg

A significant effect of the sampled ecosystems (river, igarapé and lake) on total Hg concentrations in SPM (Two-Way AONVA; $p = 0.000001$; Fig. 3A) as well as a marginally significant interaction of seasonality with the sampled ecosystems were detected (Two-Way ANOVA; $p = 0.04$; Fig. 3A).

Total Hg concentrations in the SPM were not different between the lake and igarapé in any of the evaluated hydrological periods (Fig. 3A).

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**Fig. 2.** Variation of the physical and chemical parameters with increasing distance from the Madeira River. (A) River1 and River2; (B) Igarapé1; (C) Igarapé2; (D) Lake2; (E) Lake1; (F) Lake3 sample points (Fig. 1). Logarithmic regressions explain the process satisfactorily for pH ($r^2 = 0.53; p = 0.000001$; $Y = -0.1479\ln(X) + 6.2814$), electrical conductivity ($r^2 = 0.86; p < 0.000001$; $Y = -6.352\ln(X) + 36.091$) and dissolved O$_2$ ($r^2 = 0.24; p = 0.005$; $Y = -0.4642\ln(X) + 5.6492$). The temperature did not show variations with increasing distance from the Madeira River ($r^2 = 0.02; p = 0.35$) and had median ± MAD of 27.2 ± 1.0.
On the other hand, significant differences were observed in total Hg concentrations in the SPM between the river and the igarapé-lake system in all the hydrological periods (Fig. 3A). Increasing total Hg concentrations in the SPM towards the floodplain ecosystems (river - igarapé = lake) were observed during the rising-water and early falling-water periods (Fig. 3A) and the lowest were observed in the river during the early falling-water period (5.4 ± 2.7, Fig. 3A).

3.2.2. MeHg

Sampled ecosystems (river, igarapé and lake) had different MeHg concentrations associated with SPM (Fig. 3B; Two-Way ANOVA: $p < 0.0001$). No significant direct effects of seasonality or interaction between seasonality and sampled ecosystems were detected (Two-Way ANOVA: $p = 0.29$ and $p = 0.96$, respectively). MeHg concentrations in the SPM was different between the lake and the igarapé in each hydrological period evaluated ($p < 0.000001$; Fig. 3B).

3.2.3. δ13C signature and (C:N)a ratio of organic matter

The δ13C isotopic signature and the (C:N)a ratio of the organic matter associated with the SPM overlapped between the igarapé and the lake while they could be used to differentiate the igarapé-lake system from the river (Fig. 4A). The overlap between the igarapé and the lake represented 88% of all the isotopic and elemental area covered by the igarapé and 43% of all the isotopic and elemental area covered by the lake (Fig. 4A).

The δ13C signature of the organic matter associated with the SPM from the river had higher values (heavier isotopic signature, i.e., higher 13C content related to 12C content), while smaller values (lighter isotopic signature, i.e., lower 13C content related to 12C content) were observed in the igarapé-lake system (Fig. 4A). The (C:N)a ratio presented slightly smaller values in the river compared to the igarapé-lake system (Fig. 4A).
3.2.4. MeHg and total Hg associations with geochemical supports

MeHg concentrations in the SPM were associated with the (C:N)\text{a} ratio and the $\delta^{13}$C of organic matter also in SPM (Fig. 5). Through multivariate regression ($r^2 = 0.79; p < 0.0001$) it is possible to predict higher MeHg concentrations in SPM with higher organic matter degradation (higher (C:N)\text{a} ratio values) and lighter isotopic signature (lower $\delta^{13}$C values) of organic matter (Fig. 5).

MeHg concentrations in the SPM were associated with the oxygenation level of the water column and its acidity (Fig. 6A). Through multivariate regression ($r^2 = 0.80; p < 0.0001$) it was possible to predict higher MeHg concentrations in SPM with lower the oxygenation (lower dissolved O$_2$ values) and higher acidity (lower pH values) of the water column (Fig. 6A).

MeHg concentrations in SPM were also associated with the SO$_4^{2-}$ concentrations in the water column (Fig. 7A; Supplementary material 3). Through exponential regression ($r^2 = 0.73; p < 0.0001$) it was possible to predict higher MeHg concentrations with lower the SO$_4^{2-}$ concentrations in the water column (Fig. 7A).

Total Hg concentrations in SPM were associated with total Fe concentrations also in SPM (Fig. 7B). Through quadratic regression ($r^2 = 0.57; p < 0.0001$) it was possible to predict higher total Hg concentrations in SPM with higher Fe concentrations up to a limit of 50 $\mu$g g$^{-1}$ dry wt. After this limit, a decrease in total Hg concentrations in the SPM with increasing Fe concentrations was observed.

3.3. Dissolved matter

3.3.1. Total Hg

A marginally significant effect of the interaction of sampled ecosystems (river, igarapé and lake) and seasonality on total Hg concentrations in the dissolved matter was detected (Fig. 3C; Two-Way ANOVA: $p = 0.02$).

The only seasonal difference detected in total Hg concentrations in dissolved matter, although marginally significant, was in the igarapé ($p = 0.03$), with lower values in the rising-water period (121.2 ± 41.7; Fig. 3C) compared to the late falling-water period (310.5 ± 158.0; Fig. 3C).

Spatially, total Hg concentrations in the dissolved matter from the igarapé were significantly lower (121.2 ± 41.7; Fig. 3C) than those observed in the lake during the rising-water period (256.1 ± 101.7; Fig. 3C; $p = 0.03$).

3.3.2. $\delta^{13}$C signature and (C:N)\text{a} ratio of organic matter

The $\delta^{13}$C isotopic signature and the (C:N)\text{a} ratio of the organic matter associated with the dissolved matter were overlapped between the igarapé and the lake, while they did allow for differentiation of the igarapé-lake system from the river (Fig. 4B). The overlap between the igarapé and the lake represented 62% of all the isotopic and elemental area covered by the igarapé and 46% of all the isotopic and elemental area covered by the lake (Fig. 4B).

The $\delta^{13}$C signature of the organic matter associated with the dissolved matter in the river presented higher values (heavier isotopic signature), while smaller values (lighter isotopic signature) were observed in the igarapé-lake system (Fig. 4B). The (C:N)\text{a} ratio presented slightly smaller values in the river compared to the igarapé-lake system (Fig. 4B).

Fig. 5. Multivariate regression of MeHg concentration in the SPM with the carbon and nitrogen elemental composition ((C:N)\text{a} ratio) and carbon-13 ($\delta^{13}$C) isotopic signature also in SPM. The regression plane is represented by the transparent gray form and was constructed with the following equation: $Z = e^{-0.4130X + 0.5861Y - 16.1855}$ ($r^2 = 0.79; p = 0.00000001$). If you are unable to view this graph interactively, click here.

Fig. 6. (A) Multivariate regression of MeHg concentration in the SPM with water column oxygenation (dissolved O$_2$) and its acidity (pH). The regression plane is represented by the transparent gray form and was constructed with the following equation: $Z = e^{-1.6392X - 0.0771Y + 11.8969}$ ($r^2 = 0.80; p = 0.000001$). If you are unable to view this graph interactively, click here. (B) Multivariate regression of MeHg concentration in the sediment profile with the carbon and nitrogen elemental composition ((C:N)\text{a} ratio) and carbon-13 ($\delta^{13}$C) isotopic signature. The regression plane is represented by the transparent gray form and was constructed with the following equation: $Z = 4.67X - 0.95Y + 157.52$ ($r^2 = 0.93; p = 0.004$). If you are unable to view this graph interactively, click here.
3.4. Sediment profile

3.4.1. Total Hg and MeHg

Total Hg and MeHg concentrations increased with depth (Supplementary material 4). The lowest total Hg concentration was observed in the surface layer (0 cm; 24.6 ng g⁻¹) while the highest was observed at the greatest depth (12 cm; 51.9 ng g⁻¹; Supplementary material 4).

The lowest MeHg concentration was observed in the first 4 cm (0.6 ng g⁻¹; Supplementary material 4) while the highest concentration was observed at the greatest depth (12 cm; 3.2 ng g⁻¹; Supplementary material 4). The lowest %MeHg was also observed in subsurface (4 cm, 1.8%; Supplementary material 4) while the highest %MeHg was determined at the greatest depth (12 cm; 6.2%; Supplementary material 4).

3.4.2. MeHg associations with geochemical supports

MeHg concentrations in the sediment were associated with the (C: N)ₐ ratio and the δ¹³C of organic matter also in the sediment (Fig. 6B). Through multivariate regression (r² = 0.93; p = 0.004) it was possible to predict higher MeHg concentrations in the sediment profile with higher degradation of organic matter (higher (C:N)ₐ values) and heavier isotopic signature (higher δ¹³C values; Fig. 6B).

3.5. Aquatic macrophytes

3.5.1. Total Hg and MeHg

The highest %MeHg were observed in the roots among all aquatic macrophyte tissues evaluated (%MeHg: 20.9–33.7; Fig. 8). The highest MeHg concentrations observed in E. azuera and E. crassipes were in the roots (7.5 and 6.3 ng g⁻¹, respectively), while Oryza sp. showed higher MeHg concentrations in leaves (0.9 ng g⁻¹). The highest total Hg concentrations observed in E. azuera and E. crassipes were in the roots (30.3 and 22.3 ng g⁻¹, respectively), while Oryza sp. showed higher MeHg concentrations in stems (18.6 ng g⁻¹).

MeHg concentration ratios between leaves and roots of macrophytes E. crassipes, E. azuera and Oryza sp. were 0.16, 0.04 and 1.6, respectively. For total Hg, the ratios were 0.49, 0.78 and 8.41, respectively.

3.5.2. δ¹³C signature and (C:N)ₐ ratio of organic matter

The δ¹³C signature and (C:N)ₐ ratio of the macrophyte tissues were within the range described for plants with C₃ metabolism previously analyzed in the Amazon region (Fig. 4C). Comparatively, the δ¹³C signature of the dissolved matter had a broader distribution than that from aquatic macrophytes, overlapping with signatures of Amazonian soils under the influence of plants with C₄ metabolism (Fig. 4C). The coverage of SPM δ¹³C signature was similar to dissolved matter, although with (C:N)ₐ ratios typically from phytoplankton reported for the Amazon region (Fig. 4C). The sediment showed (C:N)ₐ ratios with intermediate values between SPM and the area covered by aquatic macrophytes and dissolved matter (Fig. 4C).

4. Discussion

4.1. The physical and chemical parameters transition from Madeira River to Cuniã igarapé-lake system

The pH and dissolved O₂ decreased with increasing distance from the Madeira River in relation to the floodplain (Fig. 2), due to: (1) decreasing O₂ physical diffusion to the water column, considering the transition from a lotic and turbulent flow to a lentic one; and (2) organic matter leaching from adjacent forest soils to the igarapé-lake aquatic system (Roulet et al., 1999, 2001; Maia et al., 2009) that increases biochemical O₂ demand for decomposition and generates acidity and anoxia as a result (Aprile and Darwich, 2013). All these changes are, in the first instance, a consequence of the water type change (Sioli, 1967) from the Madeira River (white water) to the igarapé-lake system (black water). The higher (C:N)ₐ ratio observed in the igarapé-lake system compared to the river indicates higher organic matter degradation (Fig. 4A and B). Moreover, the (C:N)ₐ ratio and δ¹³C of the dissolved matter covers Amazonian soils under the influence of plants with C₄ metabolism (Fig. 4C).

The decreasing electrical conductivity observed for the same spatial context (Fig. 2) is related to three aspects. First is the progressive SPM sedimentation along the river-lake transect (Supplementary material 5; Sioli, 1967; Aprile and Darwich, 2013): the SPM transported by the Madeira River is essentially mineral (Gibbs, 1967), able to adsorb ions and to promote their co-precipitation. Hydrodynamically, the winding igarapé course (Fig. 1) promotes the sedimentation of particles (Santos and Ribeiro, 1988; Supplementary material 5). Second, organic matter leaching from adjacent forest soils (Fig. 4C) enhances the complexation of the available ions (Bezerra et al., 2009). Finally, the aquatic macrophytes present along the river-lake transect (personal
observation) act as geochemical barriers, absorbing the ions from the water column (Aprile and Darwich, 2013).

Seasonally, the highest dissolved O2 concentrations observed in the late falling-water period compared to the other periods may reflect (1) higher photosynthetic activity in this period and phytoplankton growth, which saturates the water column with O2, although the SPM concentration was higher in the late falling-water period in the igarapé-lake system compared to the other hydrological periods evaluated (Supplementary material 5; pb0.05), which, in turn, may have restricted partially the light penetration in the water column; (2) higher O2 exchange with the atmosphere due to higher water column surface/volume ratio that, in turn, is due to lower water column depth during the late falling-water period (Supplementary material 2); (3) lower microbial activity that decreases the biochemical oxygen demand. Indeed, the δ13C signature is lighter in the igarapé-lake system (Fig. 4A and B) compared to the river and with (C:N)α ratios typically from phytoplankton reported for the Amazon region (Fig. 4C). Caraballo et al. (2014) also observed a more oxygenated water column during the falling-water period, studying a floodplain under the influence of the Negro and Solimões rivers, highlighting the daily variation in oxygenation, with peaks in the epilimnion between 10:00 a.m. and 3:00 p.m. precisely when the measurements of this study were made. On the other hand, Brito et al. (2017), studying a floodplain under the influence of the Solimões River, observed a more oxygenated and homogenized water column during the low-water period compared to the rising-water and high-water period.

4.2. Spatial-temporal dynamics of total Hg in SPM and dissolved matter

The highest total Hg concentrations observed in the lake during the rising-water period (Fig. 3A) reflect the drag of particles caused by the entrance of river water into the igarapé-lake system as well as its progressive sedimentation towards the lake (Supplementary material 5); the association of low SPM loadings with high Hg concentrations (Supplementary material 5; Fig. 3A) indicates that coarse and heavier particles (with low adsorption area) settle along the igarapé path while fine and lighter particles (with high adsorption area) remain suspended in the water column. The particles in the water column are a mixture of those from the Madeira river and those from adjacent soils of the igarapé-lake system (Aprile and Darwich, 2013). The contrasting isotopic signature between the igarapé-lake system and the river shows that the organic matter present in the water column of from adjacent soils of the igarapé-lake system (Fig. 4A and B) while the mineral particles are mostly from the Madeira River (Supplementary material 5). In addition, total Hg and total Fe association in SPM (Fig. 7B) suggest that the binding sites of the mineral fraction are close to saturation, which characterizes efficient Hg transport, as also reported by Maia et al. (2009, 2017) for the particulate fraction of the water column. In this analysis, total Fe concentrations act as a proxy for Fe-oxyhydroxides, which have a layer of organic matter on their surface that enhances the SPM affinity for Hg (Feyte et al., 2010).

In the hydrodynamic context, the lake acts as the final sedimentation site of these particles that explains the higher total Hg concentrations in this environment during the rising-water period. This pattern has also been reported by other authors that have studied a river-floodplain system in the Amazon (Roulet et al., 1999; Maia et al., 2009; Brito et al., 2017; Maia et al., 2017; Supplementary material 6).

The transition from the rising-water to the initial falling-water period did not promote any change in the total Hg concentration pattern in the river-igarapé-lake system (Fig. 3A). On the other hand, the transition from the initial falling-water to the late falling-water period promoted marginally significant effects on the total Hg concentration...
between the lake and the *igarapé* that showed higher total Hg concentrations compared to the former (Fig. 3A). The similar depth between the *igarapé* and lake in this period (Supplementary material 2) facilitates the horizontal homogenization of the water column between these two environments that may explain this change. This seasonal change in Hg concentrations between the lake and the channel that connects it to its main river was also observed by Brito et al. (2017) in the low water period, compared to the rising-water and high-water periods.

This seasonal change between the rising-water and the late falling-water period can be observed more clearly in the dissolved matter (Fig. 3C) that showed a 2.5-fold increase of the total Hg concentration in the *igarapé* compared to the lake. The dissolved matter has more horizontal mobility between water masses when compared to the SPM, since smaller and lighter particles undergo lower gravity driven sedimentation. This higher mobility is reflected in the decreasing overlap of isotopic and elemental signatures of the *igarapé*-lake system between the SPM and dissolved matter of 24% (Fig. 4A and B). On the other hand, the dissolved matter is less efficient in Hg adsorption and transport per unit of dry mass, presenting concentrations 1.75-fold lower compared to SPM.

### 4.3. Methylation in the water column of the river-*igarapé*-lake system

MeHg concentration pattern in the river-*igarapé*-lake system did not show differences among the hydrological periods evaluated (Fig. 3B). On the other hand, other authors (Kasper et al., 2014; Brito et al., 2017) have observed higher MeHg concentrations during the rising-water and high-water periods (Supplementary material 6), since a deeper water column is more susceptible to developing anoxic hypolimnion that promotes Hg methylation. In this study, the water level variation was not significant among the hydrological periods sampled (Supplementary materials 1 and 2) and can explain the similarity of MeHg concentrations.

The slightly higher MeHg concentrations in the SPM observed in the *igarapé* in all hydrological periods sampled suggest higher methylation rates in this environment compared to the lake (Fig. 3B). Brito et al. (2017), studying a floodplain connected to the Solimões River, also observed higher MeHg concentrations in the channel that connects the floodplain to the main river, but only in the low-water period, compared to the rising-water and high-water periods (Supplementary material 6). In this study, however, the distance between Cuniã Lake and the Madeira River is approximately 37 km (Fig. 1), which limits the seasonal influence of the main river on the lake and may explain the similar MeHg concentrations in the SPM among hydrological periods (Fig. 3B).

Higher %MeHg in the *igarapé* compared to the lake are reflected in the organic matter elemental composition that had slightly higher (C:N) ratios in the *igarapé* compared to the lake, indicating more degraded organic matter. This association between MeHg production and the (C:N) ratios can be directly visualized in the multiple regression model (Fig. 5), which shows an exponential increase in the MeHg concentration with the increase of (C:N) ratio. In addition, the association of higher MeHg concentrations with lighter δ13C values suggests that organic matter derived from phytoplankton (Fig. 4C) is better at promoting the methylation process than that derived from adjacent soils to the *igarapé*-lake system.

Several authors have reported that sulfate-reducing bacteria are capable of Hg methylation (King et al., 1999; Benoit et al., 2002; Muresan et al., 2008). Indeed, higher MeHg concentrations in SPM were associated with lower SO4−2 concentrations in the water column (Fig. 7A). The increase of MeHg concentrations with decreasing SO4−2 concentrations suggests the consumption of these anions by the sulfate-reducing bacteria for organic matter decomposition, together with the Hg methylation.

In addition to organic matter elemental and isotopic signature, the physical and chemical parameters variables also showed associations with MeHg concentrations (Fig. 6A). The low oxygenation and higher acidity, generated by organic matter decomposition, were exponentially associated with high MeHg concentrations (Fig. 6A). This relationship between water column oxygenation and MeHg concentrations has also been observed by other authors in Amazonian ecosystems (Muresan et al., 2008; Almeida, 2012; Kasper et al., 2014; Brito et al., 2017).

### 4.4. Hg deposition and methylation in the sediment profile

The increase of total Hg and MeHg concentrations in the sediment profile with increasing depth reinforces the depositional character that the lake has in the river-*igarapé*-lake system (Supplementary material 4). This, along with the low depth of the water column during the sampling (Supplementary material 2), also suggests that there is high sediment remobilization in its upper layers which, in turn, might be (1) driven by wind or (2) associated with the seasonal flood pulse of the Madeira River (Junk and Furch, 1985). Bastos et al. (2006) and Almeida et al. (2014), analyzing the surface sediment of several lakes adjacent to the Madeira River (Supplementary material 6), observed higher total Hg concentrations in Cuniã Lake (98.13 ± 19.50), compared to the other eight sampled lakes. These higher Hg concentrations may be associated with greater distance from the lake to the Madeira River compared to the other sampled lakes, which makes it a more efficient sedimentation and retention environment.

Increasing MeHg concentration and %MeHg with depth indicate higher methylation rates in lower sediment layers. This is due to the higher anoxia found in these layers, which favors the methylation process (Mauro et al., 1999). The multiple regression model indicated an increase in MeHg concentrations with increasing (C:N) ratio values, due to the decomposition that happens in this compartment (Fig. 6B). In the same model, increasing MeHg concentrations were associated with heavier δ13C values, that indicates that the organic matter deposited in the sediments mostly comes from the adjacent soils to the *igarapé*-lake system (Fig. 4C), which nourishes dense vegetation and arboreal trees (personal observation) with C3 photosynthetic metabolism.

### 4.5. Influence of the aquatic macrophyte biotype on the total Hg and MeHg accumulation and translocation

The highest %MeHg was observed in the aquatic macrophyte roots (Fig. 8), a microenvironment that shelters a variety of microorganisms that use the organic matter retained from the water column and exudated by these plants in anaerobic respiration, generating anoxia and acidity, which promotes Hg methylation (Mauro et al., 1999).

The aquatic macrophytes *E. crassipes* and *E. azurea* that are floating and rooted biotypes, respectively, showed high total Hg and MeHg concentrations in their roots compared to *Oryza* sp., which also is a rooted biotype. On the other hand, *Oryza* sp. was the only sampled aquatic macrophyte in which total Hg and MeHg ratios between leaf and root were higher than 1, suggesting efficient translocation of these chemical species to the aerial component of the plant. This suggests that Hg translocation may be more associated with plant species than with biotype. In addition, as aquatic macrophytes harbor diverse fauna (Sánchez-Botero et al., 2003; Aprile and Darwich, 2013) and can be consumed by them, they can act in the Hg trophic transfer to the aquatic food chain. The detection of Hg and MeHg in ingestible tissues such as the fruit of *Oryza* sp. and structures frequently accessed by floral visitors like the flowers of *E. azurea* illustrate this phenomenon.

### 5. Conclusion

Typical floodplain ecosystems, represented in this study by the *igarapé* and Cuniã Lake, had higher total Hg and MeHg concentrations in the water column (SPM and dissolved material), partially independent of seasonality, compared to the Madeira River, to which they are connected. The distance between the Madeira River and Cuniã Lake...
plays an important role in the Hg and MeHg circulation, due to process that occur along the 37 km of the Cunã igarapé. Total Hg showed higher affinity for the SPM than for the dissolved matter, indicating more efficient transport by the former.

In the igarapé-lake system, the elemental and isotopic composition of the organic matter were useful to predict MeHg concentrations, as well as the oxygenation and acidity measured in the water column, showing that physical and chemical changes between the river–fluvialplain system affect MeHg circulation and production. The inverse relationship between MeHg and SO$_4^{2-}$ suggests the action of sulfate-reducing bacteria in this process.

Total Hg and MeHg concentrations as well as $\Delta$MeHg found in the sediment profile and aquatic macrophyte tissues demonstrated Hg and MeHg transfer along the trophic chain.

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Appendix A. Supplementary data

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References


